COMPARISON OF THE EFFECTS OF LOW-DOSE VS. HIGH-DOSE AMINOPHYLLINE ON LUNG FUNCTION IN EXPERIMENTAL MECONIUM ASPIRATION SYNDROME

Due to missing information on appropriate dosing of aminophylline in meconium aspiration syndrome (MAS), this study compared effects of high-dose and low-dose aminophylline on lung function of animals with MAS. Meconium-instilled rabbits were treated by low-dose (LD, 1.0 mg/kg), or high-dose (HD, 2.0 mg/kg) aminophylline at 0.5 and 2.5 h after meconium instillation, or were left untreated. Within 5 h of oxygen ventilation, HD-aminophylline improved gas exchange, reduced pulmonary shunts and ventilatory pressures, and decreased edema formation and lung neutrophils. LD-aminophylline enhanced lung function to a lower extent than HD-aminophylline, and failed to reduce lung edema and the number of lung neutrophils. Both treatments decreased lung peroxidation, with a stronger effect of HD-aminophylline on lipid oxidation and of LD-aminophylline on protein oxidation. Tracheal reactivity to histamine decreased after HD-aminophylline, while lung tissue reactivity was more reduced after LD-aminophylline. Although LD-aminophylline showed some anti-inflammatory potential, HD-aminophylline improved most of the parameters more effectively.

Key words: airway hyperreactivity, aminophylline, inflammation, meconium aspiration, oxidative injury
INTRODUCTION

Meconium aspiration syndrome (MAS) is a major cause of respiratory morbidity and mortality in the term and post-term neonates. Aspirated meconium obstructs the airways and causes ventilation/perfusion mismatch, inflammation, alveolar exudation, surfactant dysfunction, and airway hyperreactivity (1). Due to multiple interactions between the individual pathomechanisms, MAS is often difficult to treat by 'conventional' means: airway suctioning, ventilatory support or oxygen ventilation, and administration of exogenous surfactant. Recent studies have shown that anti-inflammatory drugs, e.g., systemic (2, 3) and intratracheal (4, 5) glucocorticoids, methylxanthines (6, 7), selective phosphodiesterase (PDE) inhibitors (8, 9), antioxidants (10), etc. may improve the lung function in MAS.

Methylxanthine derivatives, particularly theophylline, are widely used in the treatment of asthma and chronic obstructive pulmonary disease (COPD) (11, 12). However, the action of theophylline is strongly dose-dependent with a rather narrow therapeutic window. Since there is no information on appropriate dosing of aminophylline in MAS, this study compared the influence of low-dose vs. high-dose aminophylline on gas exchange and inflammation in an experimental model of MAS.

MATERIAL AND METHODS

The experimental design was approved by a local Ethics Committee of the Jessenius Faculty of Medicine in Martin, Slovakia.

Meconium was collected from 20 healthy term neonates, lyophilized and stored at -20°C. Before use, meconium was suspended in 0.9% NaCl at a concentration of 25 mg/ml. Adult rabbits of 2.6 ±0.3 kg were anesthetized with intramuscular ketamine (20 mg/kg; Narkamon, Spofa, Czech Republic) and xylazine (5 mg/kg; Rometar, Spofa, Czech Republic) followed by infusion of ketamine (20 mg/kg/h). Tracheotomy was performed and catheters were inserted into a femoral artery and right atrium for sampling the blood and femoral vein to administer anesthetics. Animals were then paralyzed with pipecuronium bromide (0.3 mg/kg/30 min; Arduan, Gedeon Richter, Hungary) and subjected to a pressure-controlled ventilator (Beat-2, Chirana, Slovakia). All animals were ventilated with a frequency of 30/min, fraction of inspired oxygen (FiO₂) of 0.21, peak inspiratory pressure (PIP) to keep a tidal volume (V₉) between 7-9 ml/kg and no positive end-expiratory pressure (PEEP) at this stage of experiment. After stabilization, ventilatory parameters were recorded and samples of arterial and mixed venous blood were taken for blood gas analysis and estimation of hemoglobin (Rapidlab™348, Bayer Diagnostics, Germany). Then, 4 ml/kg of meconium suspension (25 mg/ml) was instilled into the tracheal tube. From this moment, all animals were oxygen-ventilated. Within 30 min after meconium instillation, respiratory failure developed, defined as >30% decrease in dynamic lung-thorax compliance (Cdyn) and PaO₂<10 kPa at FiO₂ 1.0. Blood samples were taken and parameters recorded again. Animals then received aminophylline (Syntophyllin, Hoechst-Biotika, Slovakia) intravenously at a low-dose (1.0 mg/kg; Mec+LD group, n=7) or high-dose (2.0 mg/kg; Mec+HD group, n=8), both two times at 0.5 h and 2.5 h after meconium instillation, or were left without treatment (Mec group, n=8). Aminophylline
was diluted with saline in ratio 1:10 and administered slowly within 5 min. All animals were oxygen-ventilated for an additional 5 h.

Tracheal airflow and $V_T$ were measured by a Fleisch head connected to a pneumotachograph. Airway pressure was registered via a pneumatic catheter placed below the tracheal tube and connected to an electromanometer. $C_{dyn}$ was calculated as a ratio between $V_T$ (adjusted per kg b.w.) and airway pressure gradient (PIP-PEEP). Mean airway pressure (MAP) was calculated as $MAP=(PIP+PEEP)/2$, oxygenation index (OI) as $OI=MAP \times \text{FiO}_2/\text{PaO}_2$, and ventilation efficiency index as $VEI=3800/(PIP-PEEP) \times$ breathing rate $\times \text{PaCO}_2$. Right-to-left pulmonary shunts were calculated by a computer program using the Fick equation (3). Central venous pressure (CVP) was registered through the catheter inserted into the right atrium, connected to an electromanometer. Samples of arterial blood were taken before meconium instillation and at 1, 3, and 5 h of the treatment and total WBC count was determined in Bürker's chamber after staining by Türck. Differential WBC count was estimated microscopically after staining by Pappenheim. At the end of experiments, animals were killed by an overdose of anesthetics and lungs and trachea were excised. Left lungs were lavaged by saline (0.9 % NaCl, 37 °C) 3 x 10 ml/kg, bronchoalveolar lavage (BAL) fluid was centrifuged at 1500 rpm for 10 min and differential WBC count in the sediment was evaluated microscopically after staining by Pappenheim. Right lungs were cut, strips of the tissue were weighed and dried at 60 °C for 24 h to determine the wet/dry weight ratio, were used for estimation of lung tissue reactivity, or were homogenized for biochemical analyses.

Concentration of theophylline was determined in the blood plasma taken at the end of experiments and was measured by EMIT immunoassay (TDM Theophylline assay, Siemens HealthCare, Germany) on biochemical analyzer ADVIA 1240 (Siemens HealthCare, Germany). Products of lipid and protein oxidation were determined in the homogenate of the right lungs. Concentration of lipid peroxidation (LPO) products (thiobarbituric acid-reactive substances, TBARS) was determined from the absorbance at 532 nm and expressed in nmol/mg protein (6). Oxidative modification of proteins demonstrated as accumulation of dityrosine and lysine-LPO products was determined by fluorescence method and expressed in arbitrary units (6). Tracheal and lung smooth muscle reactivity to cumulative doses of histamine ($10^{-8}$ to $10^{-3}$ mol/l, Sigma-Aldrich, Germany) were estimated by an in vitro method (3) and shown in grams (g) of smooth muscle tension.

Between-group differences were evaluated by ANOVA with a post-hoc LSD test, within-group differences were evaluated by Wilcoxon's test. A $P<0.05$ was considered statistically significant. Data are expressed as means ±SE.

RESULTS

Meconium instillation decreased lung compliance and elevated right-to-left pulmonary shunts within 30 min. Furthermore, it deteriorated gas exchange and increased CVP and ventilatory pressures (PIP, PEEP, MAP) compared with the values before meconium instillation (all $P<0.05$; Table 1, Fig. 1, Fig. 2). Both LD- and HD-aminophylline treatment reduced intrapulmonary shunting and CVP, enhanced gas exchange, and allowed to reduce ventilatory pressures compared with the non-treated group, with more pronounced improvement observed in the HD group (all $P<0.05$; Table 1, Fig. 1, Fig. 2). HD-aminophylline significantly reduced lung wet/dry weight ratio compared with the non-treated group (6.7 ±0.3 in Mec+HD vs. 8.0 ±0.2 in Mec group; $P<0.001$), while the decrease in wet/dry
The ratio after LD-aminophylline vs. the Mec group was non-significant (7.6 ±0.3 in Mec+LD group; P>0.05).

After an initial decrease due to meconium instillation, a total number of WBC increased at 1, 3, and 5 h in both treated groups, with a more pronounced effect in the Mec+HD group vs. both Mec+LD and Mec groups (at respective hours 2.4 ±0.4, 3.0 ±0.7, and 4.9 ±1.2 x 10^9/l in Mec+HD group vs. 1.4 ±0.1, 1.6 ±0.1, and 1.7 ±0.1 x 10^9/l in Mec+LD group, and vs. 1.3 ±0.2, 1.2 ±0.1, and 1.0 ±0.1 x 10^9/l in Mec group) (for Mec+HD vs. Mec+LD and vs. Mec P<0.01). Relative number of neutrophils in the blood was higher at the end of experiments in the Mec+HD group vs. both Mec and Mec+LD groups (both P<0.001; Fig. 3). Differential WBC count in BAL showed decreased neutrophils in the Mec+HD vs. Mec (P<0.01) and Mec+LD groups (P<0.05; Fig. 3).

Total plasma theophylline concentration was 4.9 ±0.7 µg/ml in the Mec+LD group and 8.8 ±1.4 µg/ml in the Mec+HD group (P<0.01); compared with 0.0 µg/ml
in the Mec group; both P<0.001). Treatment with both LD- and HD-aminophylline significantly decreased concentration of TBARS (marker of lipid oxidation) compared with the non-treated group (both P<0.001), with more obvious decrease in Mec+HD group, also compared with Mec+LD group (P<0.05; Fig. 4). On the other side, LD-aminophylline was more effective than HD-aminophylline in reducing the concentration of dityrosines (marker of protein oxidation) compared with the Mec group (Mec+LD vs. Mec P<0.001, Mec+HD vs. Mec P<0.01, Mec+LD vs. Mec+HD P=0.07; Fig. 4). Concentrations of lysine-LPO products were reduced comparably

Fig. 1. Right-to-left pulmonary (RLS) shunts (%) in low-dose (Mec+LD) and high-dose (Mec+HD) aminophylline-treated, and in non-treated (Mec) groups. For Mec+LD vs. Mec: a P<0.05, b P<0.01; for Mec+HD vs. Mec: c P<0.05, d P<0.01, e P<0.001; for Mec+LD vs. Mec+HD: f P<0.05, g P<0.01.

Fig. 2. Oxygenation index (OI) in low-dose (Mec+LD) and high-dose (Mec+HD) aminophylline-treated, and in non-treated (Mec) groups. For Mec+LD vs. Mec: a P<0.05, b P<0.01, c P<0.001; for Mec+HD vs. Mec: d P<0.01, e P<0.001; for Mec+LD vs. Mec+HD: f P<0.05.
by both LD- and HD-aminophylline treatments (Mec+HD vs. Mec P<0.001, Mec+LD vs. Mec P<0.001, Mec+HD vs. Mec+LD P>0.05; Fig. 4).

Cumulative doses of histamine increased the contractile responses in all groups of animals. LD-aminophylline decreased tracheal reactivity to histamine at histamine concentrations of 10⁻⁸-10⁻⁴ mol/l and HD-aminophylline at histamine concentrations of 10⁻⁷-10⁻³ mol/l compared with the Mec group, while a stronger response was observed in the Mec+HD group also compared with the Mec+LD
group at histamine concentrations of $10^{-6}$-$10^{-3}$ mol/l (all P<0.05; Fig. 5A). On the other side, lung tissue reactivity to histamine was more reduced after LD-aminophylline compared with the Mec group at histamine concentrations of $10^{-7}$ and $10^{-6}$ mol/l (both P<0.01) than by HD-aminophylline, which decreased reactivity only at one histamine concentration ($10^{-6}$) compared with the Mec group (P<0.05; Fig. 5B).

**DISCUSSION**

Administration of anti-inflammatory drugs may be beneficial in the treatment of MAS (1). In our previous experiments, aminophylline treatment reduced intrapulmonary shunting, improved gas exchange, and diminished lung inflammation (6). The mentioned study (6) was the first trial evaluating effects of aminophylline on lung function of meconium-instilled animals and there is no other information on appropriate dosing of aminophylline in MAS. Therefore, the present experiments compared the effects of low-dose and high-dose aminophylline treatment in meconium-instilled animals. Both LD- and HD-aminophylline reduced shunting and requirements for artificial ventilation, enhanced gas exchange, decreased lung edema formation and number of neutrophils in BAL associated with their increase in blood vs a non-treated group, with more pronounced improvement observed in HD group. Both treatments decreased concentrations of peroxidation products compared with
non-treated group, with stronger effect of HD-aminophylline on lipid oxidation and of LD-aminophylline on protein oxidation. Tracheal reactivity to histamine significantly decreased after HD-aminophylline, while lung reactivity was more reduced after LD-aminophylline.

Our results confirmed the previous findings (12) that the action of aminophylline, due to its therapeutically effective molecule theophylline, is dependent on its plasma concentration. High, therapeutically used, theophylline plasma concentrations (10-20 µg/ml) may cause bronchodilation, vasodilation, decreasing permeability of the vascular wall, and has several anti-inflammatory effects such as increased release of IL-10, inhibition of NF-κB, and up-take of reactive oxygen species (13). These effects are mediated largely by PDE inhibition and adenosine antagonism (13). Toxic plasma concentrations (more than 20 µg/ml) may be associated with nausea, headache and adverse effects on the gastrointestinal system (mediated via PDE inhibition) and cardiovascular system (mediated predominantly via adenosine A<sub>1</sub> receptor antagonism) (11). On the other side, low plasma concentrations (5-10 µg/ml) may show anti-inflammatory and immunomodulatory action, which is not mediated by either PDE inhibition or adenosine receptor antagonism, but by direct activation of histone deacetylase activity leading to reduced transcription of inflammatory genes (13, 14).

In our study, plasma theophylline concentration at the end of experiments, i.e., 5 h after the first dose and 3 h after the second dose of treatment, was about 5 µg/ml in LD-aminophylline group and about 9 µg/ml in HD-aminophylline group. According to the pharmacodynamics of theophylline, these data are comparable with the concentrations observed after low-dose and high-dose aminophylline treatment in humans.

Administration of HD-aminophylline resulted in more pronounced improvement in respiratory and hemodynamic parameters than LD-aminophylline. It was likely related to relaxation of smooth muscle cells leading to alleviation of meconium-induced pulmonary vasoconstriction and tracheal hyperreactivity, which may enhance the lung functions in experimental animals with MAS. In addition, HD-aminophylline reduced meconium-induced parenchymal injury and lung edema and thereby improved the gas exchange by diminishing recruitment and activation of neutrophils and the production of cytokines and reactive species. Similarly to our results, administration of aminophylline at a dose of 5 mg/kg and maintained at 0.5 mg/kg per hour ameliorated neutrophil sequestration in the lungs, decreased lipid oxidation, reduced IL-8 and TNFα, and improved pulmonary oxygenation also in patients undergoing valve replacement (15). In models of acute lung injury, pretreatment with aminophylline reduced pulmonary artery pressure, lung edema formation, and lipid peroxidation (16, 17). Similarly, HD-aminophylline (30 mg/kg) administered 80-90 min after phosgene exposure significantly reduced lipid peroxidation, lung edema, and the levels of leukotrienes (18). Antioxidant
capacity of theophylline is likely further potentiated by the presence of ethylenediamine, other component of aminophylline molecule (19).

LD-aminophylline treatment in our experiments led to a milder improvement in lung function parameters and had no significant effect on lung edema, and on the number of lung neutrophils. Similar results have been found also in rats, where aminophylline at a dose of 1 mg/kg over 30 min followed by 0.5 mg/kg/h failed to prevent endotoxemia-induced respiratory and hemodynamic manifestations of sepsis (20). However, a stronger effect of LD-aminophylline on lung tissue reactivity and dityrosine levels has been shown regarding its anti-inflammatory and anti-oxidative action at the level of lung parenchyma. Comparably, both low- and high-dose aminophylline have been effective in preventing late-phase bronchoconstriction, bronchial hyperresponsiveness and an inflammatory response in sensitized and ovalbumine-provoked rats (21). In contrast to these experimental findings, low-dose theophylline (plasma concentration 5 µg/ml) reduces influx of neutrophils (22) and eosinophils (23) into the lungs and BAL of patients with asthma and the number of neutrophils in induced sputum, concentration of IL-8, and neutrophil chemotactic responses in patients with COPD (24).

In conclusion, aminophylline acts via several pathways and we can only hypothesize what mechanisms of high-dose and low-dose aminophylline are predominantly responsible for alleviated meconium-induced inflammation, pulmonary vasoconstriction, airway hyperreactivity, and oxidative damage. HD-aminophylline enhanced respiratory parameters and diminished lung edema and several inflammation-related parameters in meconium-instilled rabbits more effectively than LD-aminophylline. However, further research is warranted to elucidate the anti-inflammatory mechanisms of LD-aminophylline.

Acknowledgements: Study was supported by projects of the Ministry of Education (Developmental Project No. VV RP UP4 and Grants VEGA No. 1/2306/05 and 1/0061/08), and by Project of the European Social Fund No. SOP LZ 2005/NP1-027, 11230100433. Authors thank D. Kuliskova, J. Neuschlova, Ing. M. Hutko, D. Durcova, and M. Repcakova for technical assistance.

Conflicts of interest: No conflicts of interest were declared in relation to this article.

REFERENCES


Received: May 6, 2008
Accepted: September 1, 2008

Author’s address: D. Mokra, Department of Physiology, Jessenius Faculty of Medicine, Comenius University, Mala Hora 4, SK-03754 Martin, Slovakia; phone/fax: +421 43 4131426; e-mail: mokra@jfmed.uniba.sk